Inocula Differences Affect In Vitro Gas Production Kinetics

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Introduction

The kinetics of gas production during ruminal fermentation may provide valuable information about feeds that can be used to formulate rations and model animal responses. However, measurement of digestion kinetics is affected by methodology, and techniques must be established that provide accurate and precise estimates of kinetic parameters. Because gas production measurements provide the opportunity to estimate the digestion kinetics of both soluble and insoluble matter in feeds, it would be desirable to use this technique on a wide variety of forages, grains, supplements, and by-product feeds. Applying an in vitro technique to such a wide variety of substrates raises questions about the type of inoculum that should be used. The objective of our study was to evaluate the effects of donor animal and its diet on the measurement of gas production kinetics using both forage and concentrate substrates.

Materials and Methods

Inocula were obtained from four lactating Holstein cows in mid lactation using a balanced 4 X 4 Latin square design with a 2 X 2 factorial arrangement of treatments. The two forage sources were alfalfa and corn silage and the two fiber levels were 24 and 32% amylase-treated neutral detergent fiber (aNDF). Rations were formulated to meet National Research Council requirements for protein and minerals using corn, soybean meal, trace-mineralized salt and mineral supplements. Rations were fed twice daily at 12 h intervals to obtain > 10% refusal. Each period of the Latin square lasted 4 weeks. On day 24, rumen contents were taken from each cow and used as an inoculum for an in vitro experiment with a standard media designed to maintain pH above 6.0 at the end of fermentation. On day 28 of each period, ruminal contents were used in a second in vitro run in which the pH of the in vitro system was matched to that of the donor.

The in vitro gas production system of Mertens and Weimer was used to obtain gas production curves for alfalfa silage, corn silage, corn, soybean meal, cotton, and mixed rations using standard media or media that had pH adjusted using citric acid. About 90 mg of dry substrate was fermented. Corn and soybean meal were ground to pass a 2-mm screen using a Wiley cutter mill. Surgical absorbent cotton was cut by hand into 15-mm squares that weighed about 80 mg. Silages were not dried, but were frozen and ground through a meat grinder with a 12-mm die which resulted in a particle size distribution similar to materials ground through a 6-mm screen using a Wiley cutter mill. Amylase-treated neutral detergent fiber (aNDF) was measured using both sodium sulfite and amylase.

Results and Discussion

The rations used in this experiment resulted in differences in ruminal pH among treatments (Table 1). In addition, the ruminal pH of individual donors varied, irrespective of the ration that they consumed. When the standard buffer was used, the pH of the in vitro system after 96 h of fermentation was slightly lower than the pre-feeding ruminal pH. The in vitro pH after 96 h was similar to post-feeding ruminal pH, when the in vitro buffer was adjusted to match in vivo pH (Table 1). The diet of the inocula donors had a significant effect on the maximum asymptotic gas production, but not the rate of fermentation as indicated by the time required to reach one-half of the maximum gas production (Table 1). Inocula from donors fed alfalfa silage based rations resulted in greater average maximal gas production for the alfalfa and corn silage substrates than did the inocula from donors fed corn silage based rations. The effect of forage source was greater when pH of the in vitro buffer was adjusted to match that of the donor, but was also evident when the standard in vitro buffer was used. Although rations containing 32% aNDF resulted in greater maximal gas production than those with 24% aNDF, the effect of fiber content was not as large as that due to forage source.

Inocula from individual cows affected both rate and extent of gas production irrespective of the buffer used in vitro (Table 2). Cow 2661, which had a low ruminal pH for all diets, consistently had the lowest maximum

gas production and shortest time to one-half maximum gas production. Conversely, cow 3807 had high maximum gas production with short time to one-half maximum. The composite inocula had the largest maximum gas production in both trials, but the time to reach one-half maximum production was among the longest in the first trial and shortest in the second trial. It appears that a composite inoculum may be most desirable to obtain maximal gas production/100 mg of dry substrate assuming that this is an indication of maximal digestion.

Conclusions

Both rations and donors provided a range in inocula characteristics that influenced gas production kinetics. Differences in gas production between cows and their diets seemed to be associated with ruminal pH. Our results indicate that pH and the microbial populations associated with them have a significant impact on digestion kinetics as measured by gas production. A composite inoculum will help to minimize variations among in vitro runs and ensure an adequate population of microorganisms for diverse substrates and pH conditions. Accurate modeling of ruminal pH and its affects on digestion must be a critical component of any system that is designed to use digestion kinetic parameters to formulate rations or predict animal responses.

Table 1. Ruminal pH and effect of feeding alfalfa (AS) or corn silage (CS) rations containing 24 or 32% aNDF to inocula donors on the maximum gas production and time required to reach one-half maximum gas production in vitro when standard or matched buffers were used.[1]

	AS32	AS24	CS32	CS24
Ruminal pH				
Pre-feeding	6.07	5.78	6.30	6.14
Post-feeding	5.78	5.52	5.53	5.37
Standard in vitro buffer				
pH after 96 h	5.94	5.83	5.84	5.80
Maximum gas, ml/100 mg DM	21.98^{a}	20.32^{ab}	19.52^{ab}	19.34^{b}
Time at 1/2 maximum, h	5.59^{a}	5.96^{a}	6.57^{a}	5.63^{a}
Matched in vitro buffer				
pH after 96 h	5.59	5.46	5.40	5.35
Maximum gas, ml/100 mg DM	19.11^{a}	16.78^{a}	11.85^{b}	11.88^{b}
Time at 1/2 maximum, h	5.13^{a}	5.54^{a}	6.92^{a}	5.04^{a}

[1]Treatments with different superscripts are different at $P < .05$.

Table 2. Differences among donor cows in maximum gas production per 100 mg of substrate and time required to reach one-half maximum gas production in vitro when standard or matched buffers were used.[1]

	Cow 749	Cow 3807	Composite	Cow 3691	Cow 2661
In vivo ruminal pH					
3 h post-feeding	5.69	5.60		5.57	5.23
Standard in vitro buffer					
In Vitro pH after 96 h	5.87^{a}	5.87^{a}	5.82^{a}	5.86^{ab}	5.81^{b}
Maximum gas, ml/100mg	19.78^{b}	20.68^{ab}	22.76^{a}	21.56^{ab}	19.10^{b}
Time at 1/2 maximum, h	6.57^{a}	6.16^{ab}	6.94^{a}	6.00^{ab}	5.03^{b}
Matched in vitro buffer[2]					
In Vitro pH after 96 h	5.54^{b}	5.47^{bc}	5.84^{a}	5.44^{cd}	5.35^{d}
Maximum gas, ml/100mg	14.97^{c}	18.52^{b}	24.33^{a}	14.47^{c}	11.66^{d}
Time at 1/2 maximum, h	5.44^{ab}	5.24^{ab}	5.33^{ab}	7.01^{a}	4.93^{b}

[1]Treatments with different superscripts are different at $P < .05$.

[2]Composite inoculum was evaluated using the standard in vitro buffer when individual cow inocula were evaluated using an in vitro buffer that was matched to the ruminal pH of the donor.